

## CLAIMS

What is claimed is:

- 5 1. A method for detecting a methylated cytosine in a template nucleic acid, the method comprising:
- (a) providing a hairpin-template complex, comprising:
- 10 (i) a hairpin nucleic acid, wherein the hairpin nucleic acid is self-complementary and has a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said recognition sequence is situated so that said cleavage site is before, at, or beyond the 3' end of the hairpin nucleic acid, and wherein said hairpin nucleic acid is a self-hybrid; and
- 15 (ii) a single-stranded template nucleic acid;
- wherein 5' end of the hairpin nucleic acid is attached to the 3' end of the single-stranded template nucleic acid;
- (b) sequencing the single-stranded template nucleic acid of the hairpin-template complex, thereby producing:
- 20 (ii) a first sequence; and
- (i) a hairpin-template-complement complex, comprising the hairpin-template complex of (a), and further comprising a synthetic nucleic acid strand complementary to the template nucleic acid, wherein the synthetic nucleic acid strand is hybridized to the template nucleic acid, and wherein the complementary nucleic acid strand is attached at its 5'
- 25 end to the 3' end of the hairpin nucleic acid;
- (c) removing the complementary nucleic acid strand from the hairpin-template-complement complex, thereby recovering the hairpin-template complex;
- (d) treating the hairpin-template complex with sodium bisulfite, thereby producing a sodium bisulfite-treated template nucleic acid;
- 30 (e) sequencing the sodium bisulfite-treated template nucleic acid of (c), thereby producing a second sequence; and

- (f) comparing the first sequence and the second sequence, where the presence of a cytosine in the second sequence indicates that the cytosine at that position is methylated;

thereby detecting a methylated cytosine in the template nucleic acid.

5

2. The method of claim 1, wherein the hairpin nucleic acid is attached to a solid substrate.

3. An addressable array comprising a hairpin-template complex, comprising:

10

- (a) a hairpin nucleic acid, wherein the hairpin nucleic acid is self-complementary and has a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said recognition sequence is situated so that said cleavage site is before, at, or beyond the 3' end of the hairpin nucleic acid, and wherein said hairpin nucleic acid is a self-hybrid, and wherein the hairpin nucleic acid is attached to a solid substrate; and

15

- (b) a single-stranded template nucleic acid, wherein the 5' end of the hairpin nucleic acid is attached to the 3' end of the single-stranded template nucleic acid.

20

4. An addressable array, comprising a plurality of the hairpin-template complexes of claim 3, wherein adjacent complexes are separated by a distance of at least 10nm.

5. The addressable array of claim 4, wherein the complexes are separated by a distance of at least 100nm.

25

6. The addressable array of claim 4, wherein the complexes are separated by a distance of at least 250nm.

30

7. The addressable array of claim 4, wherein the density of the complexes is from  $10^6$  to  $10^9$  polynucleotides per  $\text{cm}^2$ .

8. The addressable array of claim 4, wherein the density of the complexes is from  $10^7$  to  $10^8$  molecules per  $\text{cm}^2$ .

5 9. A kit, comprising the addressable array of any of claims 3 to 8.

10. A method for detecting a methylated cytosine in a template nucleic acid, the method comprising:

(a) providing an anchor-template complex, comprising:

10 (i) a double-stranded nucleic acid anchor, wherein the double-stranded nucleic acid anchor comprises:

(A) a first end and a second end; and

(B) a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said cleavage site is situated so that said cleavage site is before, at, or beyond the 3' end of the first end of the double-stranded nucleic acid anchor; and

(ii) a single-stranded template nucleic acid;

wherein the 5' end of the first end of the double-stranded nucleic acid anchor is attached to the 3' end of the single-stranded template nucleic acid;

(b) sequencing the single-stranded template nucleic acid of the anchor-template complex, thereby producing:

(i) a first sequence; and

(ii) an anchor-template-complement complex, comprising the anchor-template complex of (a), and further comprising a synthetic nucleic acid strand complementary to the template nucleic acid, wherein the synthetic nucleic acid strand is hybridized to the template nucleic acid, and wherein the complementary nucleic acid strand is attached at its 5' end to the 3' end of the first end of the double-stranded nucleic acid anchor;

- (c) removing the complementary nucleic acid strand from the anchor-template-complement complex, thereby recovering the anchor-template complex;
- (d) treating the anchor-template complex with sodium bisulfite, thereby producing a sodium bisulfite-treated anchor-template complex;
- 5 (e) sequencing the sodium bisulfite-treated anchor-template complex of (d), thereby producing a second sequence; and
- (f) comparing the first sequence and the second sequence, where the presence of a cytosine in the second sequence indicates that the cytosine at that position in the template nucleic acid is methylated;
- 10 thereby detecting a methylated cytosine in the template nucleic acid.
11. The method of claim 10, wherein the double-stranded nucleic acid anchor is attached at its second end to a solid substrate.
- 15 12. An addressable array comprising an anchor-template complex, comprising:
- (a) a double-stranded nucleic acid anchor, wherein the double-stranded nucleic acid anchor comprises:
- (i) a first end and a second end; and
- (ii) a first restriction site for a nicking endonuclease, said restriction site
- 20 comprising a recognition sequence and a cleavage site, wherein said cleavage site is situated so that said cleavage site is before, at, or beyond the 3' end of the first end of the double-stranded nucleic acid anchor; and
- (b) a single-stranded template nucleic acid;
- 25 wherein the 5' end of the first end of the double-stranded nucleic acid anchor is attached to the 3' end of the single-stranded template nucleic acid.
13. An addressable array, comprising a plurality of the anchor-template complexes of claim 12, wherein adjacent complexes are separated by a distance of at least 10nm.
- 30 14. The addressable array of claim 12, wherein the complexes are separated by a distance

of at least 100nm.

15. The addressable array of claim 12, wherein the complexes are separated by a distance of at least 250nm.

5

16. The addressable array of claim 12, wherein the density of the complexes is from  $10^6$  to  $10^9$  polynucleotides per  $\text{cm}^2$ .

10

17. The addressable array of claim 12, wherein the density of the complexes is from  $10^7$  to  $10^8$  molecules per  $\text{cm}^2$ .

18. A kit, comprising the addressable array of claims 12 to 17.

15

19. A method for detecting a methylated cytosine in a template nucleic acid of known sequence, the method comprising:

- (a) providing a hairpin-template complex, comprising:

- (i) a hairpin nucleic acid, wherein the hairpin nucleic acid is self-complementary and has a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said recognition sequence is situated so that said cleavage site is before, at, or beyond the 3' end of the hairpin nucleic acid, and wherein said hairpin nucleic acid is a self-hybrid; and

20

- (ii) a single-stranded template nucleic acid;

wherein 5' end of the hairpin nucleic acid is attached to the 3' end of the single-stranded template nucleic acid;

25

- (b) treating the hairpin-template complex with sodium bisulfite, thereby producing a sodium bisulfite-treated template nucleic acid;
- (c) sequencing the sodium bisulfite-treated template nucleic acid of (b), thereby producing a sequence; and

- (d) comparing the sequence of (c) and the known sequence, where the presence of a cytosine in the sequence of (c) indicates that the cytosine at that position is methylated;

thereby detecting a methylated cytosine in the template nucleic acid of known sequence.

20. The method of claim 19, wherein the hairpin nucleic acid is attached to a solid substrate.

21. A method for detecting a methylated cytosine in a template nucleic acid of known sequence, the method comprising:

- (a) providing an anchor-template complex, comprising:

- (i) a double-stranded nucleic acid anchor, wherein the double-stranded nucleic acid anchor comprises:

(A) a first end and a second end; and

(B) a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said cleavage site is situated so that said cleavage site is before, at, or beyond the 3' end of the first end of the double-stranded nucleic acid anchor; and

- (ii) a single-stranded template nucleic acid;

wherein the 5' end of the first end of the double-stranded nucleic acid anchor is attached to the 3' end of the single-stranded template nucleic acid;

- (b) treating the anchor-template complex with sodium bisulfite, thereby producing a sodium bisulfite-treated anchor-template complex;

- (c) sequencing the sodium bisulfite-treated anchor-template complex of (b), thereby producing a sequence; and

- (d) comparing the sequence of (c) and the known sequence, where the presence of a cytosine in the sequence of (c) indicates that the cytosine at that position in the template nucleic acid is methylated;

thereby detecting a methylated cytosine in the template nucleic acid.

22. The method of claim 21, wherein the double-stranded nucleic acid anchor is attached at its second end to a solid substrate.

5 23. A method for detecting a methylated cytosine in a template nucleic acid of known sequence, wherein one or more of the cytosines in the template nucleic acid have been converted to uracil, the method comprising:

(a) providing a hairpin-template complex, comprising:

10 (i) a hairpin nucleic acid, wherein the hairpin nucleic acid is self-complementary and has a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said recognition sequence is situated so that said cleavage site is before, at, or beyond the 3' end of the hairpin nucleic acid, and wherein said hairpin nucleic acid is a self-hybrid; and

15 (ii) a single-stranded template nucleic acid;  
wherein 5' end of the hairpin nucleic acid is attached to the 3' end of the single-stranded template nucleic acid;

(b) sequencing the template nucleic acid, thereby producing a sequence; and

20 (c) comparing the sequence of (b) and the known sequence, where the presence of a cytosine in the sequence of (b) indicates that the cytosine at that position is methylated;

thereby detecting a methylated cytosine in the template nucleic acid of known sequence.

25 24. The method of claim 23, wherein the hairpin nucleic acid is attached to a solid substrate.

30 25. A method for detecting a methylated cytosine in a template nucleic acid of known sequence, wherein one or more of the cytosines in the template nucleic acid have been converted to uracil, the method comprising:

(a) providing an anchor-template complex, comprising:

- (i) a double-stranded nucleic acid anchor, wherein the double-stranded nucleic acid anchor comprises:
- (A) a first end and a second end; and
- (B) a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said cleavage site is situated so that said cleavage site is before, at, or beyond the 3' end of the first end of the double-stranded nucleic acid anchor; and
- (ii) a single-stranded template nucleic acid;
- wherein the 5' end of the first end of the double-stranded nucleic acid anchor is attached to the 3' end of the single-stranded template nucleic acid;
- (b) sequencing the anchor-template complex, thereby producing a sequence; and
- (c) comparing the sequence of (b) and the known sequence, where the presence of a cytosine in the sequence of (b) indicates that the cytosine at that position in the template nucleic acid is methylated;
- thereby detecting a methylated cytosine in the template nucleic acid.
26. The method of claim 25, wherein the double-stranded nucleic acid anchor is attached at its second end to a solid substrate.